

WHAT IS CLAIMED IS:

1. A prognostic method comprising:
  - (a) subjecting a sample comprising EGFR-expressing cancer cells obtained from a patient to quantitative analysis of the expression level of the RNA transcript of at least one gene selected from the group consisting of CD44v3; CD44v6; DR5; GRO1; KRT17; and LAMC2, or their product, and
  - (b) identifying the patient as likely to show resistance to treatment with an EGFR-inhibitor if the normalized expression levels of said gene or genes, or their products, are elevated above a defined expression threshold.
2. The method of claim 1 wherein the patient is identified as likely to show resistance to treatment with an EGFR-inhibitor if the expression level of the RNA transcript of LAMC2 is elevated above a defined expression threshold.
3. The method of claim 1 wherein the levels of the RNA transcripts of said genes are normalized relative to the mean level of the RNA transcript or the product of two or more housekeeping genes.
4. The method of claim 3 wherein the housekeeping genes are selected from the group consisting of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Cyp1, albumin, actins, tubulins, cyclophilin hypoxantine phosphoribosyltransferase (HRPT), L32, 28S, and 18S.
5. The method of claim 3 wherein the sample is subjected to global gene expression analysis of all genes present above the limit of detection.
6. The method of claim 5 wherein the levels of the RNA transcripts of said genes are normalized relative to the mean signal of the RNA transcripts or the products of all assayed genes or a subset thereof.

1           7.     The method of claim 6 wherein the level of RNA transcripts is determined by  
2 quantitative RT-PCR (qRT-PCR), and the signal is a Ct value.

1           8.     The method of claim 7 wherein the assayed genes include at least 50 cancer  
2 related genes.

1           9.     The method of claim 7 wherein the assayed genes includes at least 100 cancer  
2 related genes.

1           10.    The method of claim 1 wherein said patient is human.

1           11.    The method of claim 10 wherein said sample is a fixed, paraffin-embedded  
2 tissue (FPET) sample, or fresh or frozen tissue sample.

1           12.    The method of claim 10 wherein said sample is a tissue sample from fine  
2 needle, core, or other types of biopsy.

1           13.    The method of claim 10 wherein said quantitative analysis is performed by  
2 qRT-PCR.

1           14.    The method of claim 10 wherein said quantitative analysis is performed by  
2 quantifying the products of said genes.

1           15.    The method of claim 14 wherein said products are quantified by  
2 immunohistochemistry or by proteomics technology.

1           16.    The method of claim 10 wherein the EGFR-expressing cancer is selected from  
2 the group consisting of head and neck cancer, colon cancer, breast cancer, ovarian cancer,  
3 pancreatic cancer, and non-small cell lung carcinoma.

1           17.     The method of claim 16 wherein said cancer is head and neck cancer or colon  
2 cancer.

1           18.     The method of claim 10 further comprising the step of preparing a report  
2 comprising a statement whether the patient is likely to respond well to treatment with an  
3 EGFR inhibitor.

1           19.     The method of claim 10 further comprising the step of preparing a report  
2 comprising a statement whether the patient is likely to show resistance to treatment with an  
3 EGFR inhibitor.

1           20.     A method for predicting the likelihood that a patient diagnosed with an EGFR  
2 -expressing head or neck cancer will respond to treatment with an EGFR inhibitor,  
3 comprising determining the normalized level of one or more prognostic RNA transcripts or  
4 their products in a sample comprising EGFR-expressing cancer cells obtained from said  
5 patient, wherein the prognostic transcript is the transcript of one or more genes selected from  
6 the group consisting of: CD44s; CD82; CGA; CTSL; EGFRd27; IGFBP3; p27; P53; RB1;  
7 TIMP2; YB-1; A-Catenin; AKT1; AKT2; APC; Bax; B-Catenin; BTC; CCNA2; CCNE1;  
8 CCNE2; CD105; CD44v3; CD44v6; CD68; CEACAM6; Chk2; cMet; COX2; cripto; DCR3;  
9 DIABLO; DPYD; DR5; EDN1 endothelin; EGFR; EIF4E; ERBB4; ERK1; fas; FRP1;  
10 GRO1; HB-EGF; HER2; IGF1R; IRS1; ITGA3; KRT17; LAMC2; MTA1; NMYC; PAI1;  
11 PDGFA; PGK1; PTPD1; RANBP2; SPRY2; TP53BP1; and VEGFC, wherein expression of  
12 one or more of A-Catenin; AKT1; AKT2; APC; Bax; B-Catenin; BTC; CCNA2; CCNE1;  
13 CCNE2; CD105; CD44v3; CD44v6; CD68; CEACAM6; Chk2; cMet; COX2; cripto; DCR3;  
14 DIABLO; DPYD; DR5; EDN1 endothelin; EGFR; EIF4E; ERBB4; ERK1; fas; FRP1;  
15 GRO1; HB-EGF; HER2; IGF1R; IRS1; ITGA3; KRT17; LAMC2; MTA1; NMYC; PAI1;  
16 PDGFA; PGK1; PTPD1; RANBP2; SPRY2; TP53BP1; and VEGFC, or the corresponding  
17 gene product, above a defined threshold expression level indicates that the patient is likely to  
18 show resistance to treatment with an EGFR inhibitor, and expression of one or more of  
19 CD44s; CD82; CGA; CTSL; EGFRd27; IGFBP3; p27; P53; RB1; TIMP2; and YB-1, or the

20 corresponding gene product, above a defined threshold expression level indicates that the  
21 patient is likely to respond well to treatment with an EGFR inhibitor.

1           21.     The method of claim 20 comprising determining the normalized levels of at  
2     least two of said prognostic transcripts or their expression products.

1           22.     The method of claim 20 comprising determining the normalized levels of at  
2     least 5 of said prognostic transcripts or their expression products.

1           23.     The method of claim 20 comprising determining the normalized levels of all  
2     of said prognostic transcripts or their expression products.

1           24.     The method of claim 20 wherein said sample is a tissue sample.

1           25.     The method of claim 24 wherein said tissue is fixed, paraffin-embedded, or  
2     fresh, or frozen.

1           26.     The method of claim 24 wherein said tissue is from fine needle, core, or other  
2     types of biopsy.

1           27.     The method of claim 20 further comprising the step of preparing a report  
2     comprising a statement whether said patient is likely to respond well to treatment with an  
3     EGFR inhibitor.

1           28.     The method of claim 20 further comprising the step of preparing a report  
2     comprising a statement whether said patient is likely to show resistance to treatment with an  
3     EGFR inhibitor.

1           29.     A method comprising treating a patient diagnosed with an EGFR-expressing  
2     head or neck cancer and determined to have elevated normalized expression of one or more  
3     of the RNA transcripts of CD44s; CD82; CGA; CTSL; EGFRd27; IGFBP3; p27; P53; RB1;

4 TIMP2; and YB-1 genes, or the corresponding gene products in said cancer, or decreased  
5 normalized expression of one or more of the RNA transcripts of A-Catenin; AKT1; AKT2;  
6 APC; Bax; B-Catenin; BTC; CCNA2; CCNE1; CCNE2; CD105; CD44v3; CD44v6; CD68;  
7 CEACAM6; Chk2; cMet; COX2; cripto; DCR3; DIABLO; DPYD; DR5; EDN1 endothelin;  
8 EGFR; EIF4E; ERBB4; ERK1; fas; FRP1; GRO1; HB-EGF; HER2; IGF1R; IRS1; ITGA3;  
9 KRT17; LAMC2; MTA1; NMYC; PAI1; PDGFA; PGK1; PTPD1; RANBP2; SPRY2;  
10 TP53BP1; and VEGFC genes, or the corresponding gene products in said cancer, with an  
11 effective amount of an EGFR-inhibitor, wherein, for each gene, elevated or decreased  
12 normalized expression is defined by a defined expression threshold value.

1 30. The method of claim 29 wherein said patient has been determined to have  
2 elevated or decreased normalized expression of all of said RNA transcripts or the  
3 corresponding gene products.

1 31. A method for predicting the likelihood that a patient diagnosed with an EGFR  
2 -expressing colon cancer will respond to treatment with an EGFR inhibitor, comprising  
3 determining the normalized level of one or more prognostic RNA transcripts or their  
4 products in a sample comprising EGFR-expressing cancer cells obtained from said patient,  
5 wherein the prognostic transcript is the transcript of one or more genes selected from the  
6 group consisting of: Bak; Bclx; BRAF; BRK; Cad17; CCND3; CCNE1; CCNE2; CD105;  
7 CD9; COX2; DIABLO; ErbB3; EREG; FRP1; GPC3; GUS; HER2; HGF; ID1; ITGB3;  
8 PTPD1; RPLPO; STK15; SURV; TERC; TGFB2; TITF1; XIAP; CA9; CD134; CD44E;  
9 CD44v3; CD44v6; CDC25B; CGA; DR5; GRO1; KRT17; LAMC2; P14ARF; PDGFB;  
10 PLAUR; PPARG; RASSF1; RIZ1; Src; TFRC; and UPA, wherein the normalized level of  
11 one or more of CA9; CD134; CD44E; CD44v3; CD44v6; CDC25B; CGA; DR5; GRO1;  
12 KRT17; LAMC2; P14ARF; PDGFB; PLAUR; PPARG; RASSF1; RIZ1; Src; TFRC; and  
13 UPA, or the corresponding gene product, when above a defined expression threshold value,  
14 indicates that the patient is likely to show resistance to treatment with an EGFR inhibitor, and  
15 the normalized level of one or more of Bak; Bclx; BRAF; BRK; Cad17; CCND3; CCNE1;  
16 CCNE2; CD105; CD9; COX2; DIABLO; ErbB3; EREG; FRP1; GPC3; GUS; HER2; HGF;  
17 ID1; ITGB3; PTPD1; RPLPO; STK15; SURV; TERC; TGFB2; TITF1; and XIAP, or the

18 corresponding gene product, when above a defined expression threshold value, indicates that  
19 the patient is likely to respond well to treatment with an EGFR inhibitor.

1 32. The method of claim 31 comprising determining the normalized levels of at  
2 least two of said prognostic transcripts or their expression products.

1 33. The method of claim 31 comprising determining the normalized levels of at  
2 least 5 of said prognostic transcripts or their expression products.

1 34. The method of claim 31 comprising determining the normalized levels of all  
2 of said prognostic transcripts or their expression products.

1 35. The method of claim 31 wherein said sample is a tissue sample.

1 36. The method of claim 35 wherein the tissue is fixed, paraffin-embedded, or  
2 fresh, or frozen.

1 37. The method of claim 35 wherein the tissue is from fine needle, core, or other  
2 types of biopsy.

1 38. The method of claim 31 further comprising the step of preparing a report  
2 comprising a statement whether the patient is likely to respond well to treatment with an  
3 EGFR inhibitor.

1 39. The method of claim 31 further comprising the step of preparing a report  
2 comprising a statement whether the patient is likely to show resistance to treatment with an  
3 EGFR inhibitor.

1 40. A method comprising treating a patient diagnosed with an EGFR-expressing  
2 colon cancer and determined to have elevated normalized expression of one or more of the  
3 RNA transcripts of Bak; Bclx; BRAF; BRK; Cad17; CCND3; CCNE1; CCNE2; CD105;

4 CD9; COX2; DIABLO; ErbB3; EREG; FRP1; GPC3; GUS; HER2; HGF; ID1; ITGB3;  
5 PTPD1; RPLPO; STK15; SURV; TERC; TGFBR2; TITF1; and XIAP genes, or the  
6 corresponding gene products in said cancer, or decreased normalized expression of one or  
7 more of the RNA transcripts of CA9; CD134; CD44E; CD44v3; CD44v6; CDC25B; CGA;  
8 DR5; GRO1; KRT17; LAMC2; P14ARF; PDGFB; PLAUR; PPARG; RASSF1; RIZ1; Src;  
9 TFRC; and UPA genes, or the corresponding gene products, with an effective amount of an  
10 EGFR-inhibitor, wherein for each gene elevated or decreased normalized expression is  
11 determined relative to a defined expression threshold.

1 41. An array comprising polynucleotides hybridizing to the following genes: Bak;  
2 Bclx; BRAF; BRK; Cad17; CCND3; CD105; CD44s; CD82; CD9; CGA;; CTSL; EGFRd27;  
3 ErbB3; EREG; GPC3; GUS; HGF; ID1; IGFBP3; ITGB3; ITGB3; p27; P53; PTPD1; RB1;  
4 RPLPO; STK15; SURV; TERC; TGFBR2; TIMP2; TITF1; XIAP; YB-1; A-Catenin; AKT1;  
5 AKT2; APC; Bax; B-Catenin; BTC; CA9; CCNA2; CCNE1; CCNE2; CD134; CD44E;  
6 CD44v3; CD44v6; CD68; CDC25B; CEACAM6; Chk2; cMet; COX2; cripto; DCR3;  
7 DIABLO; DPYD; DR5; EDN1 endothelin; EGFR; EIF4E; ERBB4; ERK1; fas; FRP1;  
8 GRO1; HB-EGF; HER2; IGF1R; IRS1; ITGA3; KRT17; LAMC2; MTA1; NMYC;  
9 P14ARF; PAI1; PDGFA; PDGFB; PGK1; PLAUR; PPARG; RANBP2; RASSF1; RIZ1;  
10 SPRY2; Src; TFRC; TP53BP1; upa; and VEGFC, immobilized on a solid surface.

1 42. The array of claim 41 wherein said polynucleotides are cDNAs.

1 43. The array of claim 42 wherein said cDNAs are about 500 to about 5000 bases.

1 44. The array of claim 41 wherein said polynucleotides are oligonucleotides.

1 45. The array of claim 44 wherein said oligonucleotides are about 20 to 80 bases  
2 long.

1 46. The array of claim 45 which comprises about 330,000 oligonucleotides.

- 1           47.    The array of claim 41 wherein said solid surface is glass.
- 1           48.    An array comprising polynucleotides hybridizing to the following genes:  
2   CD44v3; CD44v6; DR5; GRO1; KRT17; LAMC2.
- 1           49.    An array comprising polynucleotides hybridizing to the following genes: A-  
2   Catenin; AKT1; AKT2; APC; Bax; B-Catenin; BTC; CCNA2; CCNE1; CCNE2; CD105;  
3   CD44v3; CD44v6; CD68; CEACAM6; Chk2; cMet; COX2; cripto; DCR3; DIABLO;  
4   DPYD; DR5; EDN1 endothelin; EGFR; EIF4E; ERBB4; ERK1; fas; FRP1; GRO1; HB-  
5   EGF; HER2; IGF1R; IRS1; ITGA3; KRT17; LAMC2; MTA1; NMYC; PAI1; PDGFA;  
6   PGK1; PTPD1; RANBP2; SPRY2; TP53BP1; VEGFC; CD44s; CD82; CGA; CTSL;  
7   EGFRd27; IGFBP3; p27; P53; RB1; TIMP2; and YB-1.
- 1           50.    An array comprising polynucleotides hybridizing to the following genes:  
2   CA9; CD134; CD44E; CD44v3; CD44v6; CDC25B; CGA; DR5; GRO1; KRT17; LAMC2;  
3   P14ARF; PDGFB; PLAUR; PPARG; RASSF1; RIZ1; Src; TFRC; UPA; CD44s; CD82;  
4   CGA; CTSL; EGFRd27; IGFBP3; p27; P53; RB1; TIMP2; and YB-1.
- 1           51.    The method of any one of claims 1, 20 and 31, wherein RNA is isolated from  
2   said tissue by a procedure comprising:  
3           (a)    incubating a section of said fixed, paraffin-embedded tissue specimen at a  
4   temperature of about 56 °C to 70 °C in a lysis buffer, in the presence of a protease, without  
5   prior dewaxing, to form a lysis solution;  
6           (b)    cooling the lysis solution to a temperature where the wax solidifies; and  
7           (c)    isolating the nucleic acid from said lysis solution.
- 1           52.    A kit comprising one or more of (1) extraction buffer/reagents and protocol;  
2   (2) reverse transcription buffer/reagents and protocol; and (3) qPCR buffer/reagents and  
3   protocol suitable for performing the method of any one of claims 1, 20 and 30.

1           53.    A method for amplification of a gene listed in Tables 5A and 5B by  
2 polymerase chain reaction (PCR), comprising performing said PCR by using an amplicon  
3 listed in Table 5A and 5B and a corresponding primer-probe set listed in Tables 6A-6F.

1           54.    A PCR primer-probe set listed in Tables 6A-6F.

1           55.    A PCR amplicon listed in Tables 5A and 5B.